IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellants: Laura Madison, Gjalt W. Huisman and Oliver P. Peoples

Serial No.: 09/235,875 Art Unit: 1638

Filed: January 22, 1999 Examiner: Russell Kallis

For: *TRANSGENIC SYSTEMS FOR THE MANUFACTURE OF POLY(3-HYDROXY-BUTYRATE-CO-3-HYDROXYHEXANOATE)*

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPEAL BRIEF

Sir:

This is an appeal from the final rejection of claims 1, 6, 7, 10, 14 and 16-21 in the Office Action mailed February 10, 2006, in the above-identified patent application. A Notice of Appeal was filed on July 10, 2006. An Advisory Action was mailed on February 15, 2006. A response to the Request for a pre-appeal brief was mailed on October 23, 2006.

A Petition for an Extension of Time for three months, up to and including December 10, 2006, and the appropriate fee, are enclosed with this response.

The fee for filing an Appeal Brief was previously submitted, with the Appeal Brief filed on April 20, 2005. Accordingly no fee for filing of this appeal brief should be due. It is believed that no additional fee is required with this submission. However, should an additional fee be required, the Commissioner is hereby authorized to charge the fee to Deposit Account No. 50-3129.

(1) REAL PARTY IN INTEREST

The real party in interest of this application is Metabolix, Inc., the assignee of record.

(2) RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences known to the appellant, the undersigned, or appellant's assignee which directly affects, which would be directly affected by, or which would have a bearing on the Board's decision in this appeal.

(3) STATUS OF CLAIMS ON APPEAL

Claims 1, 6, 7, 10, 14, 16-21 and 35-39 are pending and on appeal. Claims 2-5, 8, 9, 11-13, 15, and 22-34 have been cancelled. The text of each claim on appeal, as pending, is set forth in an Appendix to this Appeal Brief.

(4) STATUS OF AMENDMENTS

The claims were last amended in an amendment filed on May 31, 2006, subsequent to the final rejection mailed February 10, 2006. In the advisory action mailed on June 15, 2006, the Examiner indicated that this amendment would be entered. Claim 34 was cancelled in the Amendment filed on February 12, 2001. Claims 2-5, 32 and 33 were cancelled in the Amendment filed on July 29, 2002. Claims 8, 9, 11-13, 22-27 and 31 were cancelled in an Amendment filed on March 10, 2003. Claim 15 was cancelled in the Amendment filed on November 15, 2005. New claims 35-39 were added in the Amendment filed on May 31, 2006.

(5) SUMMARY OF THE CLAIMED SUBJECT MATTER

Independent Claim 1 defines a method for the biological production of polyhydroxyalkanoate containing 3-hydroxyhexanoate, which includes

providing

genetically engineered bacteria expressing a 3-ketothiolase gene encoding an enzyme that converts butyryl-CoA and acetyl-CoA to 3-ketohexanoyl-CoA (see at least page 11, lines 19-24; page 12, lines 22-26; page 13, lines 20-26), a reductase gene that encodes an acetoacetyl-CoA reductase enzyme that converts 3-ketohexanoyl-CoA to 3-hydroxyhexanoyl-CoA (see at least page 12, lines 25-26; page 21, lines 18-21), and a gene that encodes a polyhydroxyalkanoate (PHA) polymerase that polymerizes 3-hydroxybutyryl CoA and 3-hydroxyhexanoyl-CoA (see at least page 21, lines 11-15; Examples 2, 3, and 5), where the enzymes are expressed in a sufficient amount to produce polyhydroxybutyrate-co-3-polyhydroxyhexanoate, wherein the bacteria can utilize butanol or butyrate and the bacteria will produce polyhydroxybutyrate-co-3-hydroxyhexanoate is produced (please see page 5, lines 10-11).

The PHA polymerase gene can be incorporated into the bacterial chromosome, as defined by dependent claim 6 (see at least page 6, lines 20-21 and 26-27).

The bacteria can be *E. coli, Klebsiella, Ralstonia, Alcaligenes, Pseudomonas,* or *Azotobacter*, as defined by claim 14.

As defined by dependent claim 7, the -PHA polymerase gene can be obtained from *Aeromonas caviae, Comamonas testosteroni, Thiocapsia pfenigii, Chromatium vinosum, Bacillus cereus, Nocardia carolina, Nocardia salmonicolor, Rhodococcus ruber, Rhodococcus rhodocrous,* or *Rhodospirilum rubrum* (page 3, line 19-25; page 10, line 28 to page 11, line 2; page 12, lines 1-15). In one embodiment, the bacteria express three enzymes from *C. asetobutylicum* that form butyryl-CoA (as defined by dependent claim 17), a thiolase specific for 3-ketohexanoyl-CoA, a reductase specific for 3-ketohexanoyl-CoA, and a PHA polymerase that accepts both 3-hydroxybutyryl-CoA and 3-hydroxyhexanoyl-CoA (see at least page 12, lines 23-

28). (original claims 22-26, page 6, lines 4-8, page 14, lines 5-14, Figure 5, and page 12, lines 1-15).

The bacteria may express a gene encoding a D-specific enoyl-CoA hydratase as defined by dependent claim 16 (page 5, lines 20-25; page 23, lines 26-29) (See Figure 5).

Claim 18 defines the bacteria as expressing one or more fatty acid biosynthetic enzymes. These bacteria may be engineered or selected to express a gene encoding a 3-hydroxyacyl-ACP-coenzyme A transferase as defined by dependent claim 10 (page 5, lines 20-25; page 15, lines 18-23). The fatty acid bioxynthetic enzymes can convert 3-hydroxyacyl-ACP to 3-hydroxyacyl-CoA (claim 19) and can include 3-hydroxyacyl-ACP-coenzyme-A transferase, acyl-ACP thioesterase, and/or acyl-CoA syntase (claims 20 and 21). These enzymes may be from *E. coli* (claim 35), from *Nocardia salmonicolor* (claim 37) or from the *Pseudomonas putida* FaoAB complex (claim 39). These enzymes may form a complex (claim 36). The enzymes may epimerize S-3-hydroxyhexanoyl-CoA to R-3-hydroxyhexanoyl-CoA (claim 38). See original claims 22-26, page 6, lines 4-8, page 14, lines 5-14, figure 5, page 12, lines 1-15.

(6) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

i) whether claims 1, 7, 16 and 18-21 are novel as required by 35 U.S.C. §102(b) over Fukui, et al., *J. Bacteriol.*, 179:4821-4830 (1997) ("Fukui")

(ii) whether claims 1, 6-7, 16 and 18-21 are non-obvious as required by 35 U.S.C. §103(a) over Fukui in view of U.S. Patent No. 5,470,727 to Mascarenhas, et al. ("Mascarenhas") and further in view of Schubert, et al., *J. Bacteriol.* 170(12):5837-5847 (1988) ("Schubert").

(iii) whether claims 1, 7, 10 and 18-21 are non-obvious as required by 35 U.S.C. § 103(a) over Timm, et al., *Applied and Environmental Microbiology*, 56(11):3360-67 (1990) ("Timm")

in view of Fukui and further in view of Hoffmann, et al., *FEMS Microbiology Lett.*, 184:253-59 (2000) ("Hoffmann").

(iv) whether claims 1, 7, 14 and 16-21 are non-obvious as required by 35 U.S.C. § 103(a) over Schubert, in view of Fukui and further in view of Boynton, et al., *J. of Bacteriology* 178(11):3015-3024 (1996) ("Boynton") and Feigenbaum, et al., *PNAS*, 74(2):492-495 (1977) ("Feigenbaum").

It is understood that claims 35-39 have also been rejected (see Advisory Action dated June 15, 2006), but the examiner has failed to state why these claims are rejected. The decision of the Panel Decision from the Pre-Appeal Brief Review dated October 23, 2006, also fails to state the reasons for rejecting these claims. Accordinglyl these claims are discussed below assuming that each of the foregoing grounds of rejection had been applied to claims 35-39.

(7) ARGUMENTS

(a) The Claimed Invention

Polyhydroxyalkanoates (PHAs) are natural, thermoplastic polyesters and can be processed by traditional polymer techniques for use in an enormous variety of applications, including consumer packaging, disposable diaper linings and garbage bags, food and medical products. Several factors are critical for economic biological production of PHAs, including substrate costs, fermentation time, and efficiency of downstream processing.

The production of PHAs containing 3-hydroxy-co-hydroxyhexanoate (3H-co-HH) in known biological systems is inefficient. However, the Appellants have discovered that microorganisms, such as *E. coli*, which do not normally produce PHAs and have not previously been described as producing 3-hydroxyhexanoate (3HH), can be genetically engineered to produce PHAs by the introduction of a PHA synthase gene which encodes an enzyme that

accepts C6 substrates and optionally, additional enzyme encoding genes, such as genes encoding 3-ketothiolase, acetoacetyl-CoA reductase, 3-ketoacyl-CoA reductase, enoyl-CoA hydratase and/or 3-hydroxyacyl-ACP-coenzymeA transferase which provide the appropriate substrates for the polymerase to make polymer. The genes are preferably selected on the basis of the substrate specificity of their encoded enzymes for the production of polymers containing 3-hydroxyhexanoate ("3HH") as well as 3-hydroxybutyrate ("3HB). The various pathways that can be utilized to produce PHAs are shown in Figures 2-5 of the application. These vary in what substrates can be utilized, and therefore in the resulting polymers.

The Appellants have also discovered that biological systems for the production of PHAs containing 3-hydroxy-co-hydroxyhexanoate (3H-co-HH) can be improved by genetically engineering these organisms to produce the co-monomer 3-hydroxyhexanoic acid from cheaper feed stocks, such as butyrate or butanol, or directly from glucose by incorporating genes encoding enzymes which can channel cellular intermediates to butyryl-CoA, thereby improving the economics of PHA production using transgenic organisms. Even a very slight difference in the economy of production of the polymers can make a large difference in the economics, when considering the amount of polymer that can be produced by fermentation. The assignee, Metabolix, has a partnership with ADM to utilize huge fermentation reactions to use these engineered organisms to make polymer from cheap feedstocks. Enzyme activities desirable for conversion of metabolic intermediates into *R*-3-hydroxyhexanoyl-CoA, include butyryl-CoA dehydrogenase activity and acyl CoA:ACP transferase activity. The latter conversion is catalyzed either by a single protein or by a combination of thioesterase and acyl-CoA synthase activities. The flux of normal cellular metabolites to 3-hydroxyhexanoate is redirected via one or more of three different pathways. These three pathways generate 3-hydroxyhexanoate, either (1)

using a butyrate fermentation pathway, for example using enzymes from *Clostridium acetobutylicium* (Figure 3), (2) using fatty acid biosynthetic enzymes, for example from *E. coli* (Figure 5), or (3) using a fatty acid oxidation complex, for example, from *Pseudomonas putida* (Figure 4). In a preferred embodiment, *E. coli* is engineered to synthesize PHBH from either inexpensive carbohydrate feedstocks such as glucose, sucrose, xylose and lactose or mixtures of such carbohydrates and fatty acids as the only carbon source by introducing genes encoding enzymes that convert cellular metabolites to 3-hydroxyhexanoyl-CoA. It is crucial that the expression of all the genes involved in the pathway be adequate for efficient PHA synthesis in recombinant bacterial strains.

An example of a biosynthetic pathway that results in *R*-3-hydroxyhexanoyl-CoA formation involves the elongation of butyryl-CoA to 3-ketohexanoyl-CoA which can subsequently be reduced to the monomer precursor, as shown in Figure 3. Butyryl CoA is formed by butyrate fermenting organisms such as *C. acetobutylicum* in a four step pathway from acetyl CoA. Elongation of butyryl CoA to 3-ketohexanoyl CoA is catalyzed by a thiolase. The complete pathway thus involves (1) the PHB biosynthetic thiolase, (2) the three enzymes from *C. acetobutylicum* that form butyryl CoA, (3) a second thiolase, specific for 3-ketohexanoyl CoA, (4) a reductase specific for this substrate, and (5) a PHB polymerase that accepts both 3-hydroxybutyryl CoA and 3-hydroxyhexanoyl CoA.

In *P. putida* , monomers for PHA biosynthesis are derived from the fatty acid oxidation pathway when alkanes or oxidized alkanes are provided as carbon and energy source. The intermediate in this pathway that is channeled to PHA biosynthesis is *S*-3-hydroxyacyl CoA (preferentially C8 and C10) which undergoes epimerization by the FaoAB complex to the *R*-isomer. The combined action of epimerase and PHA polymerase provides C6 to C14 monomers

for PHA. Consequently, a combination of this epimerase and a 3-hydroxyhexanoyl CoA accepting PHA polymerase provides the biosynthetic capability to synthesize PHBH from fatty acids in transgenic organisms, as shown by Figure 4. Mixtures of fatty acids and carbohydrates that are useful feedstocks for fermentative production as the 3HB monomer can be derived from acetyl CoA, where the 3HH component is from fatty acids.

P. putida and *P. aeruginosa* synthesize PHAs composed of medium-chain length 3-hydroxy fatty acids when grown on sugars. The predominant monomer in these PHAs is 3-hydoxydecanoate. A similar pathway can be engineered for the synthesis of PHBH in recombinant microorganisms such as *E. coli, R. eutropha* and *P. putida*, as shown by Figure 5. In addition to a polymerase that accepts the 3-hydoxybutyryl CoA and 3-hydroxyhexanoyl CoA precursors, an enzymatic activity that converts 3-hydroxyacyl ACP into 3-hydroxyacyl CoA or 3-ketoacyl ACP into 3-ketoacyl CoA is required. Deregulation of fatty acid biosynthesis and increased activity of this pathway subsequently provides the substrate for PHBH formation. The critical enzymatic activity in this pathway is the conversion of the 3-hydroxyacyl ACP to the CoA derivative. Thioesterases and acyl CoA synthases can accomplish this step. Alternatively, acyl ACP:CoA transferase can be used to facilitate this step in the PHA pathway.

In summary, there are a variety of complex pathways that can be utilized to provide bacteria with cheap substrate to make polymers including 3HH and 3HB. Appellants have worked through these pathways to identify the necessary enzymes (and therefore genes) and made organisms that express these enzymes so that the substrates will be converted as necessary and polymer produced. This is demonstrated in the application in the examples. Example 2 at pages 21-22 shows PHBH synthesis in *E. coli* from butyrate. Example 3 at pages 22-23 shows PHBH synthesis using the butyrate pathway. Example 4 at pages 23-24 shows PHBH synthesis

in *E. coli* using a fatty acid oxidation pathway. Example 5 at pages 24-25 shows production of PHBH from butanol in *E. coli*. Example 6 at pages 25-26 shows PHBH synthesis in *E. coli* using a fatty acid biosynthetic pathway. These examples demonstrate not only that appellants have reduced to practice the claimed subject matter, but showed that these pathways can be manipulated so that the necessary enzyme is expressed at an appropriate time and in the required amount, to process substrate for the next step in the reaction, to yield a PHBH polymer. This is neither disclosed by nor obvious from the prior art.

(b) Rejections Under 35 U.S.C. § 102

The Legal Standard

For a rejection of claims to be properly founded under 35 U.S.C. § 102, it must be established that a prior art reference discloses each and every element of the claims. *Hybritech Inc v Monoclonal Antibodies Inc*, 231 USPQ 81 (Fed. Cir. 1986), *cert. denied*, 480 US 947 (1987); *Scripps Clinic & Research Found v Genentech Inc*, 18 USPQ2d 1001 (Fed. Cir. 1991). The Federal Circuit held in *Scripps*, 18 USPQ2d at 1010:

Invalidity for anticipation requires that all of the elements and limitations of the claim are found within a single prior art reference. *There must be no difference* between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention. (Emphasis added)

A reference that fails to disclose even one limitation will not be found to anticipate, even if the missing limitation could be discoverable through further experimentation. As the Federal Circuit held in *Scripps, Id.*:

[A] finding of anticipation requires that all aspects of the claimed invention were already described in a single reference: a finding that is not supportable if it is necessary to prove facts

beyond those disclosed in the reference in order to meet the claim limitations. The role of extrinsic evidence is to educate the decision-maker to what the reference meant to persons of ordinary skill in the field of the invention, not to fill in the gaps in the reference.

Analysis

Claims 1, 7, 16 and 18-21 were rejected as anticipated by Fukui.

Fukui

Fukui discloses production of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) by the β-oxidation pathway, by providing substrates of *C6 or longer fatty acids, specifically hexanoate and octanoate*. This is inherently different since the pathways and enzymes required to arrive at the PHBH are different since the substrates are different. These are not interchangeable.

Moreover, Fukui only looks at PHA-negative mutants of *A. eutrophus* and *P. putida*, organisms that normally make PHB. One does not know all of the enzymes that are expressed by these organisms or what defect has interrupted polymer production. Complementation studies are not predictive of one's ability to engineer a pathway into an organism which does not normally make polymer at all, such as *E. coli*.

Claims 1, 7 and 16

Claims 1 and 7 define a method for the biological production of PHA containing 3-hydroxyhexanoate that requires bacteria engineered with enzyme(s) to generate 3-ketohexanoyl-CoA from butyryl-CoA and acetyl-CoA. The method provides genetically engineered bacteria expressing a 3-ketothiolase gene which encodes an enzyme capable of converting butyryl-CoA and acetyl-CoA to 3-ketohexanoyl-CoA. The bacteria also encode an acetoacetyl CoA reductase gene that encodes an enzyme that converts the 3-ketohexanoyl-CoA to 3-hydroxyhexanoyl-CoA, and a polyhydroxyalkanoate polymerase that polymerizes 3-hydroxybutyryl-CoA and 3-

hydroxyhexanoyl-CoA. The method ensures that the enzymes are expressed in sufficient amounts to produce polyhydroxybutyrate-co-3-hydroxyhexanoate, and provides substrates sequentially utilized by each enzyme in the pathway, as well as grows the bacteria under conditions wherein polyhydroxybutyrate-co-3-hydroxyhexanoate is produced.

Fukui appears to disclose providing a thiolase, reductase and polymerase. Fukui does not provide the enzymes for utilization of butanol or butyrate.

Claim 16 is dependent on claim 1 and requires that the bacteria express a gene encoding D-specific enoyl-CoA hydratase. There is no reference to a D-specific enoyl-CoA hydratase in the bacteria making PHA in Fukui, only a comment that expression of ORF3 in *E. coli* showed (R)-enoyl-coenzyme A hydratase activity.

It is clear that this method is very different from that disclosed in Fukui since different pathways and enzymes are utilized. There are many pathways that can ultimately produce PHAs, as described in the specification and Figures 1-5, and different carbon sources that can be utilized by the PHA-producing organism . The method disclosed by Fukui utilizes the pathway shown in the specification in Figure 4 (β oxidation), which is also shown in Fukui (Figure 4, page 4829). The bacteria used to produce the PHAs are grown on fructose, gluconate, hexanoate, or octanoate (all C6 or C8 substrates) as a carbon source (Fukui, page 4823, column 2). This is different from the claimed method which utilizes short carbon chain substrates. The claimed method provides transgenic microorganisms that produce the co-monomer 3-hydroxyhexanoic acid from cheaper shorter carbon chain feedstock such as butyrate or butanol (please see the specification at page 5, lines 6-10). Fukui does not disclose the claimed method.

Contrary to the Examiner's assertion, a conclusion cannot be drawn using the disclosure in Schubert, that *R. eutropha* employed in Fukui for the production of polyhydroxybutyrate-co-3-

hydroxyhexanoate inherently contains a 3-ketothiolase gene which encodes an enzyme that can convert butyryl-CoA and acetyl-CoA to 3-keto-hexanoyl-CoA as required by the claimed method. The Examiner cited Schubert as evidence that *R. eutropha* comprises an endogenous 3-keto-thiolase gene (phbA). However, there is no mention in Schubert of any enzyme in *R. eutropha,* catalyzing the formation of 3-keto-hexanoyl-CoA from butyryl-CoA and acetyl-CoA.

"In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original). MPEP Section 2112 clearly states that the fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) (reversed rejection because inherency was based on what would result due to optimization of conditions, not what was necessarily present in the prior art); *In re Oelrich*, 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981).

The claimed method requires the thiolase be able to catalyze an elongation reaction that results in the production of 3-ketohexanoyl CoA from butyryl-CoA and acetyl-CoA. Schubert, in Figure 2 on page 5845 clearly shows the pathway for PHB synthesis and the related reaction steps in *A. eutrophus* (now *R. eutropha*), showing the formation of acetoacetate from acetyl-CoA by β-ketothiolase. The Examiner has provided no basis in fact or technical reasoning for the conclusion that the β-ketothiolase from *R. eutropha* disclosed in Schubert has the ability to catalyze the condensation of butyryl-CoA and acetyl-CoA to form 3-ketohexanoyl-CoA, or that every thiolase is capable of catalyzing the condensation of any two acyl-CoA molecules. For the

forgoing reasons, a conclusion cannot be drawn that a thiolase capable of catalyzing the condensation of butyryl-CoA and acetyl-CoA is inherently present in *R. eutropha*.

te from butyryl-CoA and acetyl-CoA using a polyhydroxyalkanoate from *Aeromonas caviae*

Therefore claims 1, 7 and 16 are novel over Fukui.

Claims 10, 18-21 and 35-39

Claim 18 is dependent on claim 1, and requires the bacteria express one or more fatty acid biosynthetic genes. Claim 19 is dependent on claim 18, and require the fatty acid biosynthetic enzymes convert 3-hydroxyacyl-ACP to 3-hydroacyl-CoA. Claim 20 is dependent on claim 19 and requires that enzymes are selected from the group consisting of 3-hydroxyacyl-ACP-coenzyme-A transferase, acyl-ACP thioesterase, and acyl-CoA synthase. Claim 21 is dependent on claim 20 and requires that the enzymes are acyl ACP thioesterase and acyl-CoA synthase. Claims 35-39 depend from claim 18 and further specify the enzymes.

As discussed above, Fukui does not disclose a method of production of PHBH from butyryl-CoA and acetyl CoA nor the production of a bacterial that can produce PHBH from short chain carbons. Accordingly, the methods of claims 10, 18-21 and 35-39 are novel.

Fukui does not disclose the use of the fatty acid biosynthetic pathway for the production of the co-monomer needed for polyhydroxybutyrate-co-3-hydroxyhexanoate synthesis (specification, page 15, lines 4-17, and Figure 5), which provides further evidence that claims 10, 18-21 and 35-39 are novel over Fukui.

(c) Rejections Under 35 U.S.C. § 103

The Legal Standard

The U.S. Patent and Trademark Office has the burden under 35 U.S.C. § 103 to establish a *prima facie* case of obviousness. *In re Warner et al.*, 379 F.2d 1011, 154 U.S.P.Q. 173, 177

(C.C.P.A. 1967), *In re Fine,* 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598-99 (Fed. Cir. 1988). To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck,* 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

The prior art must provide one of ordinary skill in the art with the motivation to make the proposed modifications needed to arrive at the claimed invention. *In re Geiger*, 815 F.2d 686, 2 U.S.P.Q.2d 1276 (Fed. Cir. 1987); *In re Lalu and Foulletier*, 747 F.2d 703, 705, 223 U.S.P.Q. 1257, 1258 (Fed. Cir. 1984). Claims for an invention are not *prima facie* obvious if the primary references do not suggest all elements of the claimed invention and the prior art does not suggest the modifications that would bring the primary references into conformity with the application claims. *In re Fritch*, 23 U.S.P.Q.2d, 1780 (Fed. Cir. 1992). *In re Laskowski*, 871 F.2d 115 (Fed. Cir. 1989). This is not possible when the claimed invention achieves more than what any or all of the prior art references allegedly suggest, expressly or by reasonable implication.

Analysis

(i) Claims 1, 6, 7, 10, 14, 16, 18-21, and 35-39 are non-obvious over Fukui in view Mascarenhas and further in view of Schubert.

Mascarenhas

Mascarenhas discloses methods and compositions for inserting a copy of a heterologous gene into the chromosome of a host cell such as *E. coli*, through the use of a chromosomal transfer DNA, a circular DNA, non-self-replicating DNA carrying a site-specific recombination site.

Schubert

Schubert discloses the genes in *A eutrophus* which are involved in the synthesis of PHB. The heterologous expression of the *A. eutrophus* PHB synthase genes in *E. coli* and the formation of PHB granules in recombinant strains of E. coli provided some evidence that all three genes of the PHB-synthetic pathway are clustered in *A. eutrophus* (Schubert, abstract).

Fukui, Mascarenhas and Schubert in Combination

Claims 1, 6-7 and 16

As discussed above, Fukui reports on the production of polymer from C6 to C8 substrates, acknowledging that production of P3HB from shorter chain substrates is known. The problem Fukui is addressing is the production of polymer from longer chain carbon substrates, thereby not only failing to provide motivation to one of ordinary skill in the art to make a bacteria for production of polymer from short carbon chain sources, but teaching away from doing so. It is well established that for a reference to make obvious the claimed subject matter, the references must not only disclose the claimed elements but the motivation to combine as appellants have done, with a reasonable expectation of success.

Fukui does not disclose bacteria encoding genes that enable the production of the 3-hydroxyhexanoate co-monomer from butyryl-CoA and acetyl CoA. Neither Mascarenhas nor Schubert makes up for this deficiency. Mascarenha teaches integration of a heterologous gene in the chromosome but not one that works in a pathway with other enzymes so it is not predictive of success with the claimed polymerase gene. Schubert works with defective mutants and complementation, not cloning of genes from multiple pathways that are inserted into another organism. Therefore, a combination of all three references does not recite each and every element of the claims. Furthermore, a skilled artisan would have no motivation to combine Fukui which discloses the production of PHAs from long carbon chain sources such as fructose, gluconate and octanoate, using PHA synthase from *A. caviae*, with Schubert, to create bacteria that can utilize short carbon chain substrates, nor would one have any expectation of success if one did so. The claimed method requires that the bacteria express a thiolase that can convert butyryl-Co and acetyl-CoA to 3-ketohexanoyl-CoA. None of the references cited by the Examiner disclose a thiolase with such an enzyme activity. Although Fukui shows a proposed pathway for the production of 3-hydroxyhexanoyl-CoA intermediate (Fukui, Figure 4), it is clear that this step does not involve a thiolase. Nowhere in Schubert is it disclosed that the thiolase from *A. eutropha* is capable of catalyzing the condensation of butyryl-CoA and acetyl-CoA. Thus, a skilled artisan would have no motivation to combine these references to define a method for PHA production from butyryl-CoA and acetyl-CoA, with any expectation of success. Mascarenhas does not make up for the deficiencies, since Mascarenhas merely discloses the expression of heterologous DNA in a host cell.

Therefore, claims 1, 6-7 and 16 are not obvious over Fukui, in view of Mascarenhas and further in view of Schubert

Claims 10, 18-21 and 35-39

As discussed above, none of the cited prior art disclose a method of making PHAs using butyryl-CoA and acetyl-CoA as intermediates, nor a thiolase that can catalyze condensation of these substrates in a pathway to produce PHA. Furthermore, neither Fukui, Mascarenhas nor Schubert disclose or suggest the inclusion of fatty acid biosynthetic pathway enzymes in a microorganism for the purpose of PHA synthesis from short carbon chain substrates. A skilled artisan combining Fukui, Mascarenhas and Schubert would therefore not arrive at the method as defined by claims 10, 18-21 and 35-39. Therefore, claims 10, 18-21 and 35-39 are not obvious over Fukui in view of Mascarenhas and further in view of Schubert

(ii) Claims 1, 7, 10 and 18-21 are not obvious over Timm, in view of Fukui and further in view of Hoffmann

Timm

Timm discloses transforming *Pseudomonas aeruginosa* with a polymerase (*phbC*) gene from *A. eutrophus* (*R. eutropha*). When grown on gluconate as the carbon source, these bacteria were able to accumulate a polyester with 3-hydroxydecanoate as the main constituent, with 3-hydroxyhexanoate, 3-hydroxyoctanoate and 3-hydroxy-dodecanoate as minor constituents of the polymer (Timm, abstract). If the cells were grown with octanoate as the main carbon source, a polymer consisting of 3-hydroxybutyrate, 3-hydroxyhexanoate, 3-hydroxyoctanoate and 3-hydroxy-decanoate accumulated (Timm, page 3361, results section). However, the first paragraph of the Discussion of Timm states "...the PHA synthase of *A. eutrophus* exhibits a preference for CoA derivatives of hydroxyalkanoic acids with four and five carbon atoms and **does not accept** longer derivatives as substrates", *thereby specifically excluding the claimed*

subject matter. Based on this, Timm does not disclose a suitable polymerase to produce polymer incorporating hydroxyhexanoate.

Appellants' specification also states that the polymerase from *R. eutropha* does not polymerize 3-hydroxyhexanoyl-CoA. See page 11, lines 5-9, which reads "The PHB polymerase from *R. eutropha* is highly specific for the 3-hydroxybutyryl CoA monomer and shows only 7.5% activity towards 3-hydroxyvaleryl CoA. No activity with 3-hydroxyhexanoyl CoA or longer 3-hydroxyacyl CoA's was detected in *in vitro* studies (Haywood, et al., *FEMS Microbiol. Lett.*, 57:1 (1989))."

Table 1 of Timm shows that the control strain *P. aeruginosa* PAC1 synthesizes PHA containing 3-hydroxyhexanoate *before* the PHB-synthetic genes of *A. eutrophus* are introduced (*P. aeuruginosa* PAC1 (pVK101::PP1)- see page 3361, Results Section, lines 2-4). Therefore, the ability of this strain to produce PHA containing 3-hydroxyhexanoate is due to endogenous enzymes expressed by the strain and **not** from the expression of the *A. eutrophus* genes.

Hoffmann

Hoffmann discloses the identification of a protein ($phaG_{pa}$) from *P. aeruginosa* which shares 57% identity with (R)-3-hydroxydecanoyl-ACP:CoA transacylase in *P. putida*, which enabled PHA accumulation from fatty acids in $phaG_{pa}$ mutants, suggesting this protein exhibits 3-hydroxydecanoyl-ACP:CoA transacylase activity.

Timm, Fukui and Hoffmann in combination

Claims 1, 7, 10, 18-21 and 35-39

Timm does not disclose or suggest the claimed method. Timm discloses a method that results in a polymer with at least four different monomer constituents and which does not include a PHA polymerase that can incorporate hydroxyhexanoate except when grown on gluconate.

The only conclusion that can be drawn from Timm is that the bacteria used are able to generate medium chain length intermediates such as 4-hydroxyhexanoate from gluconate and octanoate carbon sources used. The method defined by claims 1, 7, 10 and 18-21 requires that the bacteria express a thiolase that can convert butyryl-Co and acetyl-CoA to 3-ketohexanoyl-CoA for the subsequent production of the co-monomer for polyhydroxybutyrate-co-3-hydroxyhexanoate. There is no disclosure in Timm that the PHB-synthetic genes from *A. eutrophus* have the ability to catalyze the condensation of butyryl-CoA and acetyl-CoA. A skilled artisan would know that just because an enzyme has thiolase activity does not confer on it the ability to catalyze any and every reaction involving the condensation of any two acyl-CoA's.

As previously discussed, Fukui does not remedy this deficiency, nor does Hoffmann. The combination of these references does not lead to the claimed subject matter. Therefore, the combination of Timm, Fukui, and Hoffman does not recite each and every claim limitation and so cannot in combination make obvious the claims. Therefore, claims 1, 7, 10 and 18-21 are not obvious over Timm in view of Fukui and Hoffman.

(iii) Claims 1, 7, 14 and 16-21 are not obvious over Schubert in view of Fukui, and further in view of Boynton and Feigenbaum.

Boynton

Boynton discloses the cloning of β-hydroxybutyryl-CoA dehydrogenase and butyryl-CoA dehydrogenase from *C. actobutylicum*.

Feigenbaum

Feigenbaum discloses isolation of a multi-enzyme complex of fatty oxidation from *E. coli*.

Schubert, Fukui, Boynton and Feingenbaum in combination

Schubert discloses the genes in *A eutrophus* which are involved in the synthesis of PHB. The heterologous expression of the *A. eutrophus* PHB synthase genes in *E. coli* and the formation of PHB granules in recombinant strains of *E. coli* provide evidence that all three genes of the PHB-synthetic pathway are clustered in *A. eutrophus*. Schubert provides the pathway for PHB synthesis and the related reaction steps in *A. eutrophus* (Schubert, page 5845, FIG 2). It is clear from the discussion in Schubert that the thiolase activity referred to is in connection with the condensation of two acetyl-CoA units to form acetoacetyl-CoA as shown in FIG 2. Schubert does not disclose a method for production of polyhydroxybutyrate-co-3-hydroxyhexanoate.

Fukui discloses the production of polyhydroxybutyrate-co-3-hydroxyhexanoate by the β-oxidation pathway by providing substrates C6 or longer, such as hexanoate and octanoate. As depicted in Figure 4 (Fukui, page 4829), the pathway by which the 3-hydroxyhexanoate is generated according to Fukui does not involve butyryl-CoA and acetyl-CoA condensation. Fukui does not disclose the production of a PHA by a transgenic organism expressing an enzyme capable of catalyzing the condensation of butyryl-CoA and acetyl-CoA. Neither Boynton, nor Feingenbaum make up for this deficiency. The combination of Schubert, Fukui, Boynton and Feigenbaum does not recite all of the claim limitations, nor make obvious, the claimed subject matter under 35 U.S.C. 103 (a).

Furthermore, Schubert does not disclose or suggest that β-ketothiolase from *A. eutrophus* can catalyze the condensation of acetyl-CoA and butyryl-CoA. A skilled artisan would therefore not be motivated to combine Schubert with Fukui and further with Feigenbaum or Boynton, with any expectation of success. Therefore, claims 1, 7, 14 and 16-21 are not obvious over Schubert in view of Fukui and further in view of Feigenbaum or Boynton.

(d) Summary and Conclusion

For a reference to anticipate under 102 (b), it must recite **each and every** claim limitation. Fukui does not meet this requirement. Fukui fails to explicitly disclose each claim limitation. Moreover, Fukui does not inherently disclose the claimed subject matter. The examiner has failed to provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art. The Examiner has provided no basis in fact or technical reasoning, why a skilled artisan, reading Schubert, would conclude that the β-ketothiolase of Schubert would catalyze the condensation of butyryl-CoA and acetyl-CoA as required by the claimed method.

35 U.S.C. 103 is very clear: the prior art must disclose the claimed elements and the prior art must provide the motivation to combine as applicant has done, with a reasonable expectation of success.

Appellants are the first to show genes from two separate pathways, those involved in production of substrate from short carbon chain sources as well as those involved in production of a polymer, into an organism and demonstrate a successful outcome. None of the cited art leads one of skill in the art to utilize genes from two pathways, substrate formation and polymer production, as appellants' claim.

The Examiner has cited no art disclosing a method of making polyhydroxybutyrate-co-3-hydroxyhexanoate by providing genetically engineered bacteria expressing a 3-ketothiolase gene that converts butyryl-CoA and acetyl-CoA to 3-ketohexanoyl-CoA. The Examiner has cited no art disclosing any enzyme with thiolase activity capable of catalyzing the condensation of any two acyl-CoA's. The Examiner has cited no art disclosing a β-ketothiolase from *A. eutrophus* that can catalyze the condensation of butyryl-CoA and acetyl-CoA.

Therefore, the cited art cannot make obvious the claimed method.

For the foregoing reasons, Appellants submit that claims 1, 6, 7, 10, 14, 16-21, and 35-39 are novel and non-obvious.

Respectfully submitted,

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(8) Claims Appendix: Claims On Appeal

1. A method for the biological production of polyhydroxyalkanoate containing 3-hydroxyhexanoate comprising

providing genetically engineered bacteria expressing a 3-ketothiolase gene encoding an enzyme that converts butyryl-CoA and acetyl-CoA to 3-ketohexanoyl-CoA, a reductase gene that encodes an acetoacetyl-CoA reductase enzyme that converts 3-ketohexanoyl-CoA to 3-hydroxyhexanoyl-CoA, and a gene that encodes a polyhydroxyalkanoate polymerase that polymerizes 3-hydroxybutyryl-CoA and 3-hydroxyhexanoyl-CoA, wherein the enzymes are expressed in a sufficient amount to produce polyhydroxybutyrate-co-3-hydroxyhexanoate, wherein the bacteria can utilize butanol or butyrate and the bacteria will produce polyhydroxybutyrate-co-3-hydroxyhexanoate.

Claims 2-5 cancelled.

6. The method of claim 1 wherein the polyhydroxyalkanoate polymerase gene is incorporated into the bacterial chromosome.

7. The method of claim 1 for producing a copolymer of 3-hydroxyhexanoate comprising providing a polyhydroxyalkanoate polymerase gene from a bacterium selected from the group consisting of *Aeromonas caviae*, *Comamonas testosteroni*, *Thiocapsia pfenigii*, *Chromatium vinosum*, *Bacillus cereus*, *Nocardia carolina*, *Nocardia salmonicolor*, *Rhodococcus ruber*, *Rhodococcus rhodocrous*, and *Rhodospirilum rubrum*.

Claims 8-9 cancelled.

10. The method of claim 19 wherein the bacteria further comprise a gene encoding 3-hydroxyacyl-ACP-coenzyme A transferase.

Claims 11-13 cancelled.

14. The method of claim 1 wherein the bacteria is selected from the group consisting of *E. coli, Klebsiella, Ralstonia, Alcaligenes, Pseudomonas,* and *Azotobacter*.

Claim 15 cancelled.

16. The method of claim 1 wherein the bacteria express a gene encoding a D-specific enoyl-CoA hydratase.

17. The method of claim 1 wherein the bacteria express three enzymes from *C. acetobutylicum* that form butyryl CoA.

18. The method of claim 1 wherein the bacteria express one or more fatty acid biosynthetic enzymes.

19. The method of claim 18 wherein the fatty acid biosynthetic enzymes convert 3-hydroxyacyl-ACP to 3-hydroxyacyl-CoA.

20. The method of claim 19 where the enzymes are selected from the group consisting of 3-hydroxyacyl-ACP-coenzyme-A transferase, acyl-ACP thioesterase, and acyl-CoA synthase.

21. The method of claim 20 wherein the enzymes are acyl ACP thioesterase and acyl CoA synthase.

Claims 22-34 cancelled.

35. The method of claim 18 wherein the enzymes are from *E. coli*.

36. The method of claim 18 wherein the enzymes form a complex.

37. The method of claim 18 wherein the enzymes are from *Nocardia salmonicolor*.

38. The method of claim 18 wherein the enzyme epimerizes S-3-hydroxyhexanoyl-CoA to R-3-hydroxyhexanoyl-CoA.

39. The method of claim 38 wherein the enzymes are from the *Pseudomonas putida* FaoAB complex.

(9) Evidence Appendix

None

(10) Related Proceedings Appendix

None